Serial No.: 08/475,784 Filed: June 7, 1995

Page 5

## Amendment to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

## Listing of the Claims:

- 1.-100. (Cancelled)
- 101. (Currently Amended) A composition which comprises:
  - conjugate of (·i) a GD3 lactone a) a ganglioside derivative which comprises an unaltered oligosaccharide part and an altered ceramide portion comprising an altered sphingosine base a derivative of a GD3 lactone ganglioside which GD3 lactone ganglioside comprises an unaltered sphingosine base, wherein the derivative differs from the GM2 ganglioside solely by having an altered sphingosine base which retains only C1 through C4 from the unaltered sphingosine base of the GD3 lactone ganglioside, Keyhole Limpet Hemocyanin[[;]], (ii) wherein the GD3 ·lactone ganglioside derivative is covalently bound to Keyhole Limpet Hemocyanin by a stable amine bond between the C-4 carbon of the altered sphingosine base and a nitrogen of an  $\epsilon$ aminolysyl group of Keyhole Limpet Hemocyanin;

Serial No.: 08/475,784 Filed: June 7, 1995

Page 6

b) QS-21-a saponin derivable from the bark of a Quillaja saponaria Molina tree; and

c) a pharmaceutically acceptable carrier;

wherein the amount of the conjugated GD3 lactone ganglioside derivative is an amount between about 1  $\mu g$  and about 200  $\mu g$ , the amount of QS-21 the saponin is an amount of between about 10  $\mu g$  and about 200  $\mu g$ , and the GD3 lactone: Keyhole Limpet Hemocyanin molar ratio is from 200:1 to 1400:1, the relative amounts of such conjugate and such saponin being QS-21 is effective to stimulate or enhance production in a subject of an antibody to the GD3 lactone ganglioside. [[,]]

wherein in the conjugate the ganglioside derivative is covalently bound to Keyhole Limpet Hemocyanin through a C-4 carbon of the altered sphingosine base of the altered ceramide portion of the ganglioside derivative to an c-aminolysyl group of Keyhole Limpet Hemocyanin, wherein the C-4 carbon is present in a CH<sub>2</sub> group; and.

102.-107. (Cancelled)

- 108. (Currently Amended) The composition of claim 101, wherein the amount of  $\underline{QS-21}$  the saponin is about  $\underline{100}$  50  $\mu g$ .
- 109. (Currently Amended) The composition of claim

Serial No.: 08/475,784 Filed: June 7, 1995

Page 7

101, wherein the amount of QS-21 the saponin is about 200  $\mu$ g.

- 110. (Cancelled)
- 111. (Currently Amended) The composition of claim 101 which comprises:
  - a) a conjugate of (i) a GD3 lactone ganglioside derivative which comprises an unaltered oligosaccharide part and an altered ceramide portion comprising an altered sphingosine base a derivative of a GD3 lactone ganglioside which GD3 ganglioside lactone comprises an unaltered sphingosine base, wherein the differs derivative from the GM2 ganglioside solely by having an altered sphingosine base which retains only C1 through C4 from the unaltered sphingosine base of the GD3 lactone ganglioside, and (ii) Keyhole Limpet Hemocyanin[[;]], wherein the GD3 lactone ganglioside derivative is covalently bound to Keyhole Limpet Hemocyanin by a stable amine bond between the C-4 carbon of the altered sphingosine base and a nitrogen of an εaminolysyl group of Keyhole Limpet Hemocyanin;
  - b) QS-21, a saponin derivable from the bark of a Quillaja saponaria Molina tree; and

Serial No.: 08/475,784 Filed: June 7, 1995

Page 8

c) a pharmaceutically acceptable carrier;

wherein the amount of the conjugated GD3 lactone ganglioside derivative is present in an amount of between about 1 µg and about 200 µg, the amount of the saponin QS-21 is about 100 µg, and the GD3 lactone: Keyhole Limpet Hemocyanin molar ratio is from 200:1 to 1400:1, and wherein the amount relative amounts of such conjugate and such saponin QS-21 is effective to stimulate or enhance production in a subject of an antibody to the GD3 lactone ganglioside. [[;]]

and wherein in the conjugate the ganglioside derivative is covalently bound to Keyhole Limpet Hemocyanin through a C-4 carbon of the altered sphingosine base of the altered ceramide portion of the ganglioside derivative to an c-aminolysyl group of Keyhole Limpet Hemocyanin, wherein the C-4 carbon is present in a CH<sub>2</sub> group; and.

- 112. (Currently Amended) A method of treating a subject afflicted with melanoma which comprises administering to said subject an amount of the composition of claim 111 effective to stimulate or enhance production of an antibody to <a href="the GD3">the GD3</a> ganglioside in the subject and to thereby treat said melanoma in said subject.
- 113. (Currently Amended) A method of stimulating or enhancing production of an antibody to the GD3

Serial No.: 08/475,784 Filed: June 7, 1995

Page 9

<u>lactone ganglioside</u> in a subject which comprises administering to the subject an effective amount of a composition which comprises:

- a) a conjugate of (i) a GD3 lactone ganglioside derivative which comprises an unaltered oligosaccharide part and an altered ceramide portion comprising an altered sphingosine base a derivative of a GD3 lactone ganglioside which GD3 ganglioside comprises lactone an unaltered sphingosine base, wherein the derivative differs from the GM2 ganglioside solely by having an altered sphingosine base which retains only C1 through C4 from the unaltered sphingosine base of the GD3 lactone ganglioside, and Hemocyanin[[;]], (ii) Keyhole Limpet wherein the GD3 lactone ganglioside derivative is covalently bound to Keyhole Limpet Hemocyanin by a stable amine bond between the C-4 carbon of the altered sphingosine base and a nitrogen of an  $\epsilon$ aminolysyl group of Keyhole Limpet Hemocyanin;
- b) QS-21 a saponin derivable from the bark of a Quillaja saponaria Molina tree; and
- c) a pharmaceutically acceptable carrier;

wherein the amount of the conjugated GD3 lactone

Serial No.: 08/475,784 Filed: June 7, 1995

Page 10

ganglioside derivative is an amount between about 1 µg and about 200 µg, the amount of the saponin QS-21 is an amount between about 10 µg and about 200 µg, and the GD3 lactone: Keyhole Limpet Hemocyanin molar ratio is from 200:1 to 1400:1, and the relative amounts οf conjugate and such saponin being QS-21 effective to stimulate or enhance production on a subject of an antibody to the GD3 lactone ganglioside.[[,]]

wherein in the conjugate the ganglioside derivative is covalently bound to Keyhole Limpet Hemocyanin through a C-4 carbon of the altered sphingosine base of the altered ceramide portion of the ganglioside derivative to an c-aminolysyl group of Keyhole Limpet Hemocyanin, wherein the C-4 carbon is present in a CH<sub>2</sub> group, so as to thereby stimulate or enhance production in the subject of the antibody to GD3.

- 114. (Currently Amended) A method of treating a human subject having cancer which comprises administering to the subject an effective amount of a composition which comprises:
  - a) a conjugate of (i) a GD3 lactone ganglioside derivative which comprises an unaltered oligosaccharide part and an altered ceramide portion comprising an altered sphingosine base a derivative of a GD3 lactone ganglioside which GD3

Serial No.: 08/475,784 Filed: June 7, 1995

Page 11

lactone ganglioside comprises an unaltered sphingosine base, wherein the derivative differs from the GM2 ganglioside solely by having an altered sphingosine base which retains only C1 through C4 from the unaltered sphingosine base of the GD3 lactone ganglioside, and (ii) Keyhole Limpet Hemocyanin[[;]], wherein the GD3 lactone ganglioside derivative is covalently bound to Keyhole Limpet Hemocyanin by a stable amine bond between the C-4 carbon of the altered sphingosine base and a nitrogen of an  $\epsilon$ of aminolysyl group Keyhole Limpet Hemocyanin;

- b) QS-21 a saponin derivable from the bark of a Quillaja saponaria Molina tree; and
- c) a pharmaceutically acceptable carrier;

wherein the amount of the conjugated GD3 lactone ganglioside derivative is an amount between about 1  $\mu g$  and about 200  $\mu g$ , the amount of the sapenin QS-21 is an amount of between about 10  $\mu g$  and about 200  $\mu g$ , and the GD3 lactone: Keyhole Limpet Hemocyanin molar ratio is from 200:1 to 1400:1, the relative amounts of such conjugate and such sapenin QS-21 is being effective to stimulate or enhance production in a subject of an antibody to the GD3 lactone ganglioside,

Serial No.: 08/475,784 Filed: June 7, 1995

Page 12

wherein in the conjugate the ganglioside derivative is covalently bound to Keyhole Limpet Hemocyanin through a C-4 carbon of the altered sphingosine base of the altered ceramide portion of the ganglioside derivative to an e-aminolysyl group of Keyhole Limpet Hemocyanin, wherein the C-4 carbon is present in a CH<sub>2</sub> group, so as to thereby stimulate or enhance production of an antibody to GD3 in the subject and thereby treat the cancer.

- 115. (Previously Presented) The method of claim 114, wherein the cancer is of epithelial origin.
- 116. (Previously Presented) The method of claim 114, wherein the cancer is of neuroectodermal origin.
- 117. (Currently Amended) The method of claim 116, wherein the cancer of neuroectodermal origin is a melanoma.
- 118. (Previously Presented) The method of claim 113 or 114, wherein the administering is effected at two or more sites.
- 119. (Previously Presented) The method of claim 118, wherein the administering is effected at three sites.
- 120. (Previously Presented) The method of claim 113 or 114, wherein the composition is administered subcutaneously to said subject.

Serial No.: 08/475,784 Filed: June 7, 1995

Page 13

121. (Previously Presented) The method of claim 120, wherein the composition is administered to said subject at two-week intervals.

- 122. (Previously Presented) The method of claim 120, wherein the composition is initially administered to said subject at weekly intervals.
- 123. (Currently Amended) The method of claim 113 or 114, wherein the composition to be administered is prepared prior to administration to the subject by mixing the conjugate and QS-21 the saponin.
- 124. (Currently Amended) The method of claim 123, wherein the conjugate and  $\underline{QS-21}$  the saponin are mixed on the day of administration to the subject.
- 125. (Cancelled)